UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF CHEMICAL SAFETY AND
POLLUTION PREVENTION

August 13, 2014

MEMORANDUM

Subject: Efficacy Review for Lonza Laundry Bacteriostat-Sanitizer; EPA Reg. no. 6836-63; Bardac 20-10 (EPA Reg. No. 6836-65); DB Barcode: D418742.

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Applicant: Lonza Inc,
90 Boroline Road
Allendale, NJ 07401, USA

Formulation from the Label:

	Lonza Laundry Bacteriostat-Sanitizer	Bardac 20-10
Active Ingredients	% by wt.	
Octyl decyl dimethyl ammonium chloride	25 %	5 %
Dioctyl dimethyl ammonium chloride	10 %	2 %
Didecyl dimethyl ammonium chloride	15 %	3 %
Other Ingredients	50 %	90 %
Total	100 %	100 %

I. BACKGROUND

The product, Lonza Laundry Bacteriostat-Sanitizer (EPA Reg. no. 6836-63), is EPA registered product. The registrant is submitting an amendment to add claims for non-food contact sanitizer, laundry sanitizer and residual self-sanitizing. Data generated will support the use of the following products:

Lonza Laundry Bacteriostat-Sanitizer (EPA Reg. No. 6836-63) – Primary
Bardac 20-10 (EPA Reg. No. 6836-65) – Secondary

The efficacy studies were conducted on Lonza Laundry Bacteriostat-Sanitizer to support both products. Lonza Laundry Bacteriostat-Sanitizer is a 100% repack of Bardac 2050 (EPA Reg. No. 6836-52), while Bardac 20-10 is a dilution of Bardac 2050. Studies were conducted at ATS Labs, located at 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121.

Registrant submitted data (MRID 494455-01) generated in support for residual mildew fungistatic claims against *Aspergillus niger* (ATCC 6275), *Penicillium glaucum* (Fabric Mildew Fungistatic Test in April 27, 1971) and residual self-sanitization against *Klebsiella pneumoniae* (ATCC 4352), *Staphylococcus aureus* (ATCC 6538), and *Escherichia coli* (September 24, 1971). Data were generated using Bardac 20 (EPA Reg. No. 6836-19), which is identical in composition to Lonza Laundry Bacteriostat-Sanitizer according to the registrant.

This data package identified as D418742 contained a letter from the applicant to EPA (dated February 27, 2014), EPA Form 8570-1 (Application for Pesticide), EPA Form 8570-34 (Certification with Respect to Citation of Data), EPA Form 8570-35 (Data Matrix), nine studies (MRID Nos. 4492865-01 through 492865-09), Statements of No Data Confidentiality for all nine studies, and the proposed labels.

II. USE DIRECTIONS

RESIDUAL SELF-SANITIZING
Use 12 oz. of **LONZA LAUNDRY BACTERIOSTAT-SANITIZER** per a maximum total of 60 gallons [500 lbs.] of water and a maximum of 100 pounds of fabric [dry weight]. Dilute the appropriate amount of **LONZA LAUNDRY BACTERIOSTAT-SANITIZER** in one to two gallons of water and then add this solution to the wash wheel at the beginning of the final rinse cycle. A minimum rinse cycle time of 8 minutes is required.

LAUNDRY SANITIZER:
Add [mix] 12 oz. of **LONZA LAUNDRY BACTERIOSTAT-SANITIZER** per 60 gallons of water and a maximum of 100 pounds of fabric [dry weight] to sanitize laundered fabrics in the rinse cycle. A minimum cycle time of 5 minutes is required.

LAUNDRY PRE-SOAK SANITIZER:
Add [mix] 12 oz. of **LONZA LAUNDRY BACTERIOSTAT-SANITIZER** per 60 gallons of water and a maximum of 100 pounds of fabric [dry weight] to sanitize fabrics prior to laundering. A minimum soak time of 5 minutes is required.

III. AGENCY STANDARDS FOR PROPOSED CLAIMS

Residual Sanitization Activity – Laundered Fabrics: The Agency recommends use of the American Association of Textile Chemists and Colorists (AATCC) Test Method 100 Quantitative

Procedure: Assessment of Antibacterial Finishes on Textile Materials, or the Standard Test Method for Determining the Antimicrobial Activity of Immobilized Antimicrobial Agents Under Dynamic Contact Conditions (ASTM E2149). Testing should be conducted on three samples, representing three different batches of the product, one of which should be at least 60 days old. The test microorganisms should be *Staphylococcus aureus* (ATCC 6538) and *Klebsiella pneumoniae* (ATCC 4352). Quantitative bacteriological assays should be performed at the following time intervals: 0, 30 min., 1-hr, 2-hr, 3-hr, 6-hr, and 24-hr. Consideration by the Agency can be given to fewer or different time intervals, depending on the label claims, on a case-by-case basis. **Evaluation of Self-sanitizing success.** The results should demonstrate a reduction of ≥99.9 percent (a 3-log10 reduction) in bacteria over the control count for both laundry water and fabric. This reduction should be demonstrated against each test microorganism within the contact time claimed on the label.

Laundry Sanitizer – For Use During Commercial-Industrial-Institutional Laundry Operations: The effectiveness of laundry sanitizers must be supported by data that show that the product will substantially reduce the numbers of test bacteria on fabric and in laundry water. Laundry additives may either be used as soaking treatments prior to laundering or as treatments added during laundry operations. The label must specify the type of use. Laundry additives may be recommended for household/coin-operated machine use or commercial-industrial-institutional use. The label must specify the type of use. There is a significant difference in the water to fabric ratio between these two uses, which may affect the efficacy of the product. Tests should be conducted using a simulated-use procedure such as Petrocci and Clarke's "Proposed Test Method for Antimicrobial Laundry Additives" or a simulated use study involving washing machines. Tests should be performed with each of 3 product samples, representing 3 different product lots, one of which is at least 60 days old. Tests should be conducted against *Staphylococcus aureus* (ATCC 6538) and *Klebsiella pneumoniae* (ATCC 4352). Products labeled as being suitable for hospital use must also be tested against *Pseudomonas aeruginosa* (ATCC 15442). Each product lot must be tested with 3 fabrics swatches against each of the test organisms. The method employed must include subculturing of both the fabric and the laundry water. The laundry water to media volume ratio must not exceed 1:40. Testing of a 0.5 mL sample of laundry water from the simulated washing device (or a 5 mL sample from the automatic washer) is recommended. Results from a quantitative bacteriological assay must be reported. Results must show a bacterial reduction of 99.9% over the control count for both fabric and laundry water for each organism tested. The label directions for use of laundry additives should specify the machine cycle in which the product is to be added, as well as water level, temperature, and treatment time. Compatibility of the treatment with other laundry additives should be determined in testing and addressed in labeling, when applicable. These Agency standards do not apply to sodium-calcium hypochlorites, sodium-potassium dichloro-s-triazinetriones, or trichloro-s-triazinetrione.

Note: The water to fabric ratio for household laundry operations is about 10:1. The water to fabric ratio in industrial laundering operations is about 5:1. The water to fabric ratio for high efficiency operations is about 4:1.

IV. BRIEF DESCRIPTION OF THE DATA

Note: The product lots Lot # 5935-060A, Lot # 5935-060B, Lot # 5935-060C, Lot # 5977-03F Lot # 5977-03G, and Lot # 5977-03H were tested.

1. MRID 492865-01 "Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Sanitizers (Dilutable); Test Organisms: *Enterobacter aerogenes* (ATCC 13048) and *Staphylococcus aureus* (ATCC 6538)" for Lonza Laundry Bacteriostat-Sanitizer, by Joshua Luedtke. Study conducted at ATS Labs. Study completion date – August 29, 2013. Project Number A15405.

This study was conducted against *Enterobacter aerogenes* (ATCC 13048) and *Staphylococcus aureus* (ATCC 6538). Three lots (Lot # 5977-03F Lot # 5977-03G, and Lot # 5977-03H) of the product, Lonza Laundry Bacteriostat-Sanitizer, were tested according to ATS Labs Protocol LZ01073113.NFS (copy provided). The product was diluted 1:641 defined as 1 part test substance + 640 parts 200 ppm AOAC Synthetic Hard Water (titrated 200 ppm). Tryptic Soy Agar with 5% Sheep Blood was used as agar plate medium. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Five (5) glass slide carriers per lot and per organism were inoculated with 20 µl of 48-54 hour old culture of the test organism and sprayed uniformly over the test surface (approximately 1 square inch) of the slide. The glass slide carriers were dried for 35 minutes at 35-37°C at 40% relative humidity. Each carrier was treated with 5 ml of test substance and held at room temperature (22°C) and 45% relative humidity for 5 minutes. After exposure, 20 ml of Letheen Broth with 0.28% Lecithin and 2.0% Tween 80 were added to neutralize. The vessel was shaken thoroughly. Aliquots were plated within 30 min, directly or after filtration. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were visually enumerated. Representative subcultures showing growth were subcultured, stained and/or biochemically assayed to confirm or rule out the presence of the test organism. Controls included those for purity, sterility, viability, neutralization confirmation, initial suspension, and carrier population. The reported average $\log_{10}$ per dried carrier, for the test microorganism, is: ***Enterobacter aerogenes* 7.29 and *Staphylococcus aureus* 6.64.**

2. MRID 492865-02 "Residual Self-Sanitizing Activity of Laundry Additives; Test Organisms: *Klebsiella pneumoniae* (ATCC 4352) and *Staphylococcus aureus* (ATCC 6538)" for Lonza Laundry Bacteriostat-Sanitizer, by Joshua Luedtke. Study conducted at ATS Labs. Study completion date – April 3, 2013. Project Number A14774.

This study was conducted against *Klebsiella pneumoniae* (ATCC 4352) and *Staphylococcus aureus* (ATCC 6538). Three lots (5935-060A, 5935-060B and 5935-060C) of the product, Lonza Laundry Bacteriostat-Sanitizer, were tested according to ATS Labs Protocol LZ01021313.LRES (copy provided). The product was diluted 0.05 oz. per gallon (defined as 1.417g test substance + 3,785g sterile tap water) at 5:1 (weight/weight) for Commercial, Final Rinse Applications. Laundry treatment time was 8 minutes and exposure times were 0 min, 30 min, 1 hour, 2 hours, 3 hours, 6 hours, and 24 hours. Tryptic Soy Agar with 5% Sheep Blood was used as agar plate medium. One stack of 4 swatches represented a "carrier" and one replicate was tested per test organism per lot per exposure time. Swatches were cut from dried treated fabric spindles. Each stack was inoculated with 1000 µl of 24±4 hour old culture. Following inoculation, the stack of swatches was transferred to individual, sterile 8 oz. jars. The jars were sealed and the carriers were allowed to expose at 35-37°C (35.9°C). Following exposure, 100.0 ml of Letheen Broth

with 0.07% Lecithin and 0.5% Tween 80 was added to each of the jars containing the exposed carriers. The jars were shaken for approximately one minute to release any remaining viable test organism. This neutralization step represented the 10° dilution. Serial dilutions of the neutralized solution were prepared and aliquots were plated. All subcultures were incubated for 48±4 hours at 35-37°C and stored 1-2 days at 2-8°C. Following incubation and storage, the subcultures were visually enumerated. Representative subcultures showing growth were subcultured, stained and/or biochemically assayed to confirm or rule out the presence of the test organism. Controls included those for purity, sterility, viability, neutralization confirmation, initial suspension, and carrier population. The reported average colony forming units (CFU) per ml in initial suspension control, for each test microorganism, are as follows: ***Klebsiella pneumoniae*** **2.24×10^6 and *Staphylococcus aureus* 4.2×10^5.**

Note: Protocol amendments reported in the study were reviewed.

3. MRID 492865-03 "Standard Test Method for the Evaluation of Laundry Sanitizers; Test Organisms: *Corynebacterium ammoniagenes* (ATCC 6872)" for Lonza Laundry Bacteriostat-Sanitizer, by Jill Ruhme. Study conducted at ATS Labs. Study completion date – October 1, 2013. Project Number A15527.

This study was conducted against *Corynebacterium ammoniagenes* (ATCC 6872). Two lots (Batch 5977-03F and Batch 5977-03G) of the product, Lonza Laundry Bacteriostat-Sanitizer, were tested using ATS Labs Protocol No. LZ01082113.LSAN.5 (copy provided). The product was diluted 1:640 (defined as 1 part test substance+ 639 parts 200 ppm AOAC Synthetic Hard Water (titrated 200 ppm) at 4.0:1 (weight/weight) for High Efficiency Applications. Soy Agar with 5% Sheep Blood was used as agar plate medium. Letheen Broth with 0.07% Lecithin and 0.5% Tween 80 was used as neutralizer subculturing solution. Three sterile fabric swatch carriers (1 inch by 1.5 inch), cut from the scoured fabric per product lot were inoculated with 0.03 ml of the prepared organism culture, and dried in an incubator at 35-37°C for 10 minutes, until visibly dry. After drying, the swatches were each inserted between the 6th and 7th lap of a wrapped spindle. Each spindle contained three dried, contaminated swatches. The spindles were placed in the Nalgene jars containing 60±0.1 grams the use solution and subjected to a simulated tumble-wash at 45-60 RPM for 5 minutes at 35±1°C. A 0.5 ml aliquot of the "wash" water was transferred to a vessel containing 9.5 ml of neutralizer. The fabric swatches were transferred to 10 ml neutralizer. The fabric swatches were then vortex mixed for a minimum of 10 seconds to extract fabric-bound microorganisms. The neutralizing subculture medium was vortex mixed, then serially diluted, and plated (1.0ml) in duplicate. All subcultures were incubated for 48-54 hours at 35-37°C followed by storage at 2-8°C for 1 day prior to examination. Following incubation and storage, the subcultures were then examined for the presence or absence of visible growth and standard plate count procedures were used to determine the average colony forming units per carrier. Controls included those for purity, sterility, viability, initial inoculum confirmation, carrier population, and neutralization confirmation. The reported average colony forming units (CFU) per carrier, for the test microorganism is: ***Corynebacterium ammoniagenes*** **9.77×10^6.**

Note: Protocol amendment reported in the study was reviewed.

4. MRID 492865-04 "Standard Test Method for the Evaluation of Laundry Sanitizers; Test Organisms: *Escherichia coli* O157:H7 (ATCC 35150)" for Lonza Laundry Bacteriostat-Sanitizer, by Joshua Luedtke. Study conducted at ATS Labs. Study completion date – October 2, 2013. Project Number A15526.

This study was conducted against *Escherichia coli* O157:H7 (ATCC 35150). Two lots (Batch 5977-03F and Batch 5977-03G) of the product, Lonza Laundry Bacteriostat-Sanitizer, were tested using ATS Labs Protocol No. LZ01082113.LSAN.3 (copy provided). The product was diluted 1:640 (defined as 1 part test substance+ 639 parts 200 ppm AOAC Synthetic Hard Water (titrated 200 ppm) at 4.0:1 (weight/weight) for High Efficiency Applications. Tryptic Soy Agar with 5% Sheep Blood was used as agar plate medium. Letheen Broth with 0.07% Lecithin and 0.5% Tween 80 was used as neutralizer subculturing solution. Three sterile fabric swatch carriers (1 inch by 1.5 inch), cut from the scoured fabric per product lot were inoculated with 0.03 ml of the prepared organism culture, and dried in an incubator at 35-37°C for 11 minutes, until visibly dry. After drying, the swatches were each inserted between the 6th and 7th lap of a wrapped spindle. Each spindle contained three dried, contaminated swatches. The spindles were placed in the Nalgene jars containing 60±0.1 grams the use solution and subjected to a simulated tumble-wash at 45-60 RPM for 5 minutes at 35±1°C. A 0.5 ml aliquot of the "wash" water was transferred to a vessel containing 9.5 ml of neutralizer. The fabric swatches were transferred to 10 ml neutralizer. The fabric swatches were then vortex mixed for a minimum of 10 seconds to extract fabric-bound microorganisms. The neutralizing subculture medium was vortex mixed, then serially diluted, and plated (1.0ml) in duplicate. All subcultures were incubated for 48-54 hours at 35-37°C followed by storage at 2-8°C for 2 days prior to examination. Following incubation and storage, the subcultures were then examined for the presence or absence of visible growth and standard plate count procedures were used to determine the average colony forming units per carrier. Controls included those for purity, sterility, viability, initial inoculum confirmation, carrier population, and neutralization confirmation. The reported average colony forming units (CFU) per carrier, for the test microorganism is: ***Escherichia coli* 4.90 x 10^6.**

Note: Protocol amendment reported in the study was reviewed.

5. MRID 492865-05 "Standard Test Method for the Evaluation of Laundry Sanitizers; Test Organisms: *Klebsiella pneumoniae* (ATCC 4352), *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 15442)" for Lonza Laundry Bacteriostat-Sanitizer, by Joshua Luedtke. Study conducted at ATS Labs. Study completion date – October 11, 2013. Project Number A15524.

This study was conducted against *Klebsiella pneumoniae* (ATCC 4352), *Staphylococcus aureus* (ATCC 6538), and *Pseudomonas aeruginosa* (ATCC 15442). Three lots (Batch 5977-03F, Batch 5977-03G, and Batch 5977-03F) of the product, Lonza Laundry Bacteriostat-Sanitizer, were tested using ATS Labs Protocol No. LZ01082113.LSAN.1 (copy provided). The product was diluted 1:640 (defined as 1 part test substance+ 639 parts 200 ppm AOAC Synthetic Hard Water (titrated 195 ppm) at 4.0:1 (weight/weight) for High Efficiency Applications. Tryptic Soy Agar with 5% Sheep Blood was used as agar plate medium. Letheen Broth with 0.07% Lecithin and 0.5% Tween 80 was used as neutralizer subculturing solution. Three sterile fabric swatch carriers (1 inch by 1.5 inch), cut from the scoured fabric per organism and per product lot were inoculated with 0.03 ml of the prepared organism culture, and dried in an incubator at 35-37°C for 10 minutes, until visibly dry. After drying, the swatches were each inserted between the 6th and 7th lap of a wrapped spindle. Each spindle contained three dried, contaminated swatches. The spindles were placed in the Nalgene jars containing 60±0.1 grams the use solution and subjected to a simulated tumble-wash at 45-60 RPM for 5 minutes at 35±1°C. A 0.5 ml aliquot of the "wash" water was transferred to a vessel containing 9.5 ml of neutralizer. The fabric swatches were transferred to 10 ml neutralizer. The fabric swatches were then vortex mixed for a minimum of 10 seconds to extract fabric-bound microorganisms. The neutralizing subculture medium was vortex mixed, then serially diluted, and plated (1.0ml) in duplicate. All subcultures

were incubated for 48-54 hours at 35-37°C followed by storage at 2-8°C for 1 days prior to examination. Following incubation and storage, the subcultures were then examined for the presence or absence of visible growth and standard plate count procedures were used to determine the average colony forming units per carrier. Controls included those for purity, sterility, viability, initial inoculum confirmation, carrier population, and neutralization confirmation. The reported average colony forming units (CFU) per carrier for test microorganisms are as followed: ***Klebsiella pneumoniae* 3.47 x 10^7, *Staphylococcus aureus* 4.07 x 10^6, and *Pseudomonas aeruginosa* 3.72 x 10^6.**

Note: Protocol deviation reported in the study was reviewed.

6. MRID 492865-06 "Standard Test Method for the Evaluation of Laundry Sanitizers; Test Organisms: *Listeria monocytogenes* (ATCC 19117)" for Lonza Laundry Bacteriostat-Sanitizer, by Jill Ruhme. Study conducted at ATS Labs. Study completion date – October 17, 2013. Project Number A15519.

This study was conducted against *Listeria monocytogenes* (ATCC 19117). Two lots (Batch 5977-03F and Batch 5977-03G) of the product, Lonza Laundry Bacteriostat-Sanitizer, were tested using ATS Labs Protocol No. LZ01082113.LSAN.4 (copy provided). The product was diluted 1:640 (defined as 1 part test substance+ 639 parts 200 ppm AOAC Synthetic Hard Water (titrated 200 ppm) at 4.0:1 (weight/weight) for High Efficiency Applications. Tryptic Soy Agar with 5% Sheep Blood was used as agar plate medium. Letheen Broth with 0.07% Lecithin and 0.5% Tween 80 was used as neutralizer subculturing solution. Three sterile fabric swatch carriers (1 inch by 1.5 inch), cut from the scoured fabric per product lot were inoculated with 0.03 ml of the prepared organism culture, and dried in an incubator at 35-37°C for 10 minutes, until visibly dry. After drying, the swatches were each inserted between the 6th and 7th lap of a wrapped spindle. Each spindle contained three dried, contaminated swatches. The spindles were placed in the Nalgene jars containing 60±0.1 grams the use solution and subjected to a simulated tumble-wash at 45-60 RPM for 5 minutes at 35±1°C. A 0.5 ml aliquot of the "wash" water was transferred to a vessel containing 9.5 ml of neutralizer. The fabric swatches were transferred to 10 ml neutralizer. The fabric swatches were then vortex mixed for a minimum of 10 seconds to extract fabric-bound microorganisms. The neutralizing subculture medium was vortex mixed, then serially diluted, and plated (1.0 ml) in duplicate. All subcultures were incubated for 48-54 hours at 35-37°C followed. Following incubation the subcultures were then examined for the presence or absence of visible growth and standard plate count procedures were used to determine the average colony forming units per carrier. Controls included those for purity, sterility, viability, initial inoculum confirmation, carrier population, and neutralization confirmation. The reported average colony forming units (CFU) per carrier, for the test microorganism is: ***Listeria monocytogenes* 5.62 x 10^7.**

Note: Protocol amendments and deviation reported in the study were reviewed.

7. MRID 492865-07 "Standard Test Method for the Evaluation of Laundry Sanitizers; Test Organisms: *Salmonella enterica* (ATCC 10708)" for Lonza Laundry Bacteriostat-Sanitizer, by Joshua Luedtke. Study conducted at ATS Labs. Study completion date – October 11, 2013. Project Number A15525.

This study was conducted against *Salmonella enterica* (ATCC 10708). Two lots (Batch 5977-03F and Batch 5977-03G) of the product, Lonza Laundry Bacteriostat-Sanitizer, were tested using ATS Labs Protocol No. LZ01082113.LSAN.2 (copy provided). The product was diluted 1:640 (defined as 1 part test substance+ 639 parts 200 ppm AOAC Synthetic Hard

Water (titrated 195 ppm) at 4.0:1 (weight/weight) for High Efficiency Applications. Tryptic Soy Agar with 5% Sheep Blood was used as agar plate medium. Letheen Broth with 0.07% Lecithin and 0.5% Tween 80 was used as neutralizer subculturing solution. Three sterile fabric swatch carriers (1 inch by 1.5 inch), cut from the scoured fabric per product lot were inoculated with 0.03 ml of the prepared organism culture, and dried in an incubator at 35-37°C for 10 minutes, until visibly dry. After drying, the swatches were each inserted between the 6th and 7th lap of a wrapped spindle. Each spindle contained three dried, contaminated swatches. The spindles were placed in the Nalgene jars containing 60±0.1 grams the use solution and subjected to a simulated tumble-wash at 45-60 RPM for 5 minutes at 35±1°C. A 0.5 ml aliquot of the "wash" water was transferred to a vessel containing 9.5 ml of neutralizer. The fabric swatches were transferred to 10 ml neutralizer. The fabric swatches were then vortex mixed for a minimum of 10 seconds to extract fabric-bound microorganisms. The neutralizing subculture medium was vortex mixed, then serially diluted, and plated (1.0ml) in duplicate. All subcultures were incubated for 48-54 hours at 35-37°C. Following incubation, the subcultures were then examined for the presence or absence of visible growth and standard plate count procedures were used to determine the average colony forming units per carrier. Controls included those for purity, sterility, viability, initial inoculum confirmation, carrier population, and neutralization confirmation. The reported average colony forming units (CFU) per carrier, for the test microorganism is: ***Salmonella enterica* 1.29 x 10^7.**

Note: Protocol deviation reported in the study was reviewed.

8. MRID 492865-08 "Standard Test Method for the Evaluation of Laundry Sanitizers; Test Organisms: Community Acquired Methicillin Resistant *Staphylococcus aureus* – CA-MRSA Genotype USA 300 (NARSA NRS 384)" for Lonza Laundry Bacteriostat-Sanitizer, by Gracia Schroeder. Study conducted at ATS Labs. Study completion date – October 1, 2013. Project Number A15529.

This study was conducted against Community Acquired Methicillin Resistant *Staphylococcus aureus* – CA-MRSA Genotype USA 300 (NARSA NRS 384). Two lots (Batch 5977-03F and Batch 5977-03G) of the product, Lonza Laundry Bacteriostat-Sanitizer, were tested using ATS Labs Protocol No. LZ01082113.LSAN.7 (copy provided). The product was diluted 1:640 (defined as 1 part test substance+ 639 parts 200 ppm AOAC Synthetic Hard Water (titrated 205 ppm) at 4.0:1 (weight/weight) for High Efficiency Applications. Tryptic Soy Agar with 5% Sheep Blood was used as agar plate medium. Letheen Broth with 0.07% Lecithin and 0.5% Tween 80 was used as neutralizer subculturing solution. Three sterile fabric swatch carriers (1 inch by 1.5 inch), cut from the scoured fabric per product lot were inoculated with 0.03 ml of the prepared organism culture, and dried in an incubator at 35-37°C for 10 minutes, until visibly dry. After drying, the swatches were each inserted between the 6th and 7th lap of a wrapped spindle. Each spindle contained three dried, contaminated swatches. The spindles were placed in the Nalgene jars containing 60±0.1 grams the use solution and subjected to a simulated tumble-wash at 45-60 RPM for 5 minutes at 35±1°C. A 0.5 ml aliquot of the "wash" water was transferred to a vessel containing 9.5 ml of neutralizer. The fabric swatches were transferred to 10 ml neutralizer. The fabric swatches were then vortex mixed for a minimum of 10 seconds to extract fabric-bound microorganisms. The neutralizing subculture medium was vortex mixed, then serially diluted, and plated (1.0ml) in duplicate. All subcultures were incubated for 48-54 hours at 35-37°C followed by storage at 2-8°C for 1 days prior to examination. Following incubation and storage, the subcultures were then examined for the presence or absence of visible growth and standard plate count procedures were used to determine the average colony forming units per carrier. Controls included those for purity, sterility, viability, initial inoculum confirmation,

carrier population, and neutralization confirmation. The reported average colony forming units (CFU) per carrier, for the test microorganism is: **CA-MRSA Genotype USA 300 3.55 x 10^7.**

Note: Antibiotic resistance of Community Acquired Methicillin Resistant *Staphylococcus aureus* – CA-MRSA Genotype USA 300 (NARSA NRS 384) was verified on a representative culture. An individual Mueller Hinton agar plate was streaked with the prepared culture. A control agar was prepared using *Staphylococcus aureus* (ATCC 25923) as a control organism. An Oxacillin disk was placed on each plate. The plates were incubated and, following incubation, the zone of inhibition was measured. The measurement confirmed antibiotic resistance of Community Acquired Methicillin Resistant *Staphylococcus aureus* – CA-MRSA Genotype USA 300 (NARSA NRS 384) to Oxacillin (Methicillin). See page 20 of the laboratory report.

Note: Protocol amendment reported in the study was reviewed.

9. MRID 492865-09 "Standard Test Method for the Evaluation of Laundry Sanitizers; Test Organisms: Hospital Acquired Methicillin Resistant *Staphylococcus aureus* – HA-MRSA (NARSA NRS 382)" for Lonza Laundry Bacteriostat-Sanitizer, by Gracia Schroeder. Study conducted at ATS Labs. Study completion date – October 1, 2013. Project Number A15528.

This study was conducted against Hospital Acquired Methicillin Resistant *Staphylococcus aureus* – HA-MRSA (NARSA NRS 382). Two lots (Batch 5977-03F and Batch 5977-03G) of the product, Lonza Laundry Bacteriostat-Sanitizer, were tested using ATS Labs Protocol No. LZ01082113.LSAN.6 (copy provided). The product was diluted 1:640 (defined as 1 part test substance+ 639 parts 200 ppm AOAC Synthetic Hard Water (titrated 205 ppm) at 4.0:1 (weight/weight) for High Efficiency Applications. Tryptic Soy Agar with 5% Sheep Blood was used as agar plate medium. Letheen Broth with 0.07% Lecithin and 0.5% Tween 80 was used as neutralizer subculturing solution. Three sterile fabric swatch carriers (1 inch by 1.5 inch), cut from the scoured fabric per product lot were inoculated with 0.03 ml of the prepared organism culture, and dried in an incubator at 35-37°C for 10 minutes, until visibly dry. After drying, the swatches were each inserted between the 6th and 7th lap of a wrapped spindle. Each spindle contained three dried, contaminated swatches. The spindles were placed in the Nalgene jars containing 60±0.1 grams the use solution and subjected to a simulated tumble-wash at 45-60 RPM for 5 minutes at 35±1°C. A 0.5 ml aliquot of the "wash" water was transferred to a vessel containing 9.5 ml of neutralizer. The fabric swatches were transferred to 10 ml neutralizer. The fabric swatches were then vortex mixed for a minimum of 10 seconds to extract fabric-bound microorganisms. The neutralizing subculture medium was vortex mixed, then serially diluted, and plated (1.0ml) in duplicate. All subcultures were incubated for 48-54 hours at 35-37°C followed by storage at 2-8°C for 1 days prior to examination. Following incubation and storage, the subcultures were then examined for the presence or absence of visible growth and standard plate count procedures were used to determine the average colony forming units per carrier. Controls included those for purity, sterility, viability, initial inoculum confirmation, carrier population, and neutralization confirmation. The reported average colony forming units (CFU) per carrier, for the test microorganism is: **HA-MRSA 3.72 x 10^7.**

Note: Antibiotic resistance of Hospital Acquired Methicillin Resistant *Staphylococcus aureus* – HA-MRSA (NARSA NRS 382) was verified on a representative culture. An individual Mueller Hinton agar plate was streaked with the prepared culture. A control agar was prepared using *Staphylococcus aureus* (ATCC 25923) as a control organism. An Oxacillin disk was placed on each plate. The plates were incubated and, following incubation, the zone of inhibition was

measured. The measurement confirmed antibiotic resistance of Hospital Acquired Methicillin Resistant *Staphylococcus aureus* – HA-MRSA (NARSA NRS 382) to Oxacillin (Methicillin). See page 20 of the laboratory report.

Note: Protocol amendment reported in the study was reviewed.

10. MRID 494455-01 "Efficacy Data for Lonza Inc.'s Lonza Laundry Bacteriostat-Sanitizer: Report for Fabric Fungistatic Tests [*Aspergillus niger* (ATCC 6275), *Penicillium glaucum*] - Evaluation of Antibacterial Finishes on Fabrics [*Escherichia coli* (ATCC 4352), *Staphylococcus aureus* (ATCC 6538), and *Brevibacterium ammoniagenes* (ATCC 6371)] - Detection of Antibacterial Activity of Fabrics [*Klebsiella pneumoniae, Staphylococcus aureus* (ATCC 6538), and *Escherichia coli* (ATCC 4352)]" for Bardac 20 (Lonza Laundry Bacteriostat-Sanitizer), by Richard A. Wahl. Study conducted at Biotech Control Laboratories, Inc. Study completion dates: August 8, 2014. Project Numbers BCI-0771, BCI-7171, and BCI-7217.

a) Residual Fabric Fungistatic Tests; Fabric Mildew Fungistatic Test Method, Issued February 25, 1969, Revised July 30, 1969.
Organisms: *Aspergillus niger* (ATCC 6275), *Penicillium glaucum*
Tested Concentrations: 440 ppm, 550 ppm, 880 ppm, and 1760 ppm in 5:1 water to fabric ratio after 10 minutes exposure.
Controls include but not limited to PMA laundry product (PMA Control) and Untreated Control.

b) Biological Properties: Antibacterial Finishes on Fabrics, Evaluation of: ATCC 100-1965T, Test Methods, Part II, B154-B155, volume 41, 1965.
Test Organisms: *Escherichia coli* (ATCC 4352), *Staphylococcus aureus* (ATCC 6538), and *Brevibacterium ammoniagenes* (ATCC 6371).
Tested lots: 906E, 947E, and 7268 at 175 ppm in 5:1 water to fabric ratio after 10 minutes exposure.
Contact times: 30 minutes, 1 hour, 6 hours, and 24 hours with 4 swatches for each contact time.

c) Biological Properties, Detection of Antibacterial Activity of Fabrics: Seeded Agar Plate Method: ATCC 90-1965T, B175-176, Technical Manual of the American Association of Textile Chemists and Colorists, volume 40, 1968.
Test Organisms: *Klebsiella pneumoniae*, *Staphylococcus aureus* (ATCC 6538), and *Escherichia coli* (ATCC 4352).
Tested lots: 906E, 947E, and 7268 at 175 ppm in 5:1 water to fabric ratio.
After 5 minutes contact time, 5 carriers per organism per lot.

V. RESULTS

MRID #	Organism	Lot #	Average Number of survivors	Carrier Population Count	Percent Reduction (5 min)
492865-01	*Enterobacter aerogenes* (ATCC 13048)	5977-03F	$<5.75 \times 10^2$	1.95×10^7	>99.9%
		5977-03G	$<4.57 \times 10^2$		>99.9%
		5977-03H	$<7.94 \times 10^2$		>99.9%
	Staphylococcus aureus (ATCC 6538)	5977-03F	$<2.63 \times 10^1$	4.37×10^6	>99.9%
		5977-03G	$<3.02 \times 10^1$		>99.9%
		5977-03H	$<2.51 \times 10^1$		>99.9%

MRID # 462865-02	Lot #	Exposure Time after 8 min Treatment	Average Number of survivors	Carrier Population Count	Percent Reduction
Klebsiella pneumoniae (ATCC 4352)	5935-060A	Time Zero	1.23×10^5	4.4×10^5	N/A
		30 minutes	$< 1 \times 10^2$	5.4×10^5	>99.9%
		1 hour	$< 1 \times 10^2$	4.6×10^5	>99.9%
		2 hours	$< 1 \times 10^2$	3.6×10^5	>99.9%
		3hours	$< 1 \times 10^2$	2.04×10^6	>99.9%
		6 hours	$< 1 \times 10^2$	$>3.00 \times 10^7$	>99.9%
		24 hours	$< 1 \times 10^2$	$>3.00 \times 10^7$	>99.9%
	5935-060B	Time Zero	1.15×10^5	4.4×10^5	N/A
		30 minutes	1×10^2	5.4×10^5	>99.9%
		1 hour	$< 1 \times 10^2$	4.6×10^5	>99.9%
		2 hours	$< 1 \times 10^2$	3.6×10^5	>99.9%
		3hours	$< 1 \times 10^2$	2.04×10^6	>99.9%
		6 hours	$< 1 \times 10^2$	$>3.00 \times 10^7$	>99.9%
		24 hours	$< 1 \times 10^2$	$>3.00 \times 10^7$	>99.9%
	5935-060C	Time Zero	1.26×10^5	4.4×10^5	N/A
		30 minutes	1×10^2	5.4×10^5	>99.9%
		1 hour	$< 1 \times 10^2$	4.6×10^5	>99.9%
		2 hours	$< 1 \times 10^2$	3.6×10^5	>99.9%
		3hours	$< 1 \times 10^2$	2.04×10^6	>99.9%
		6 hours	$< 1 \times 10^2$	$>3.00 \times 10^7$	>99.9%
		24 hours	$< 1 \times 10^2$	$>3.00 \times 10^7$	>99.9%
Staphylococcus aureus (ATCC 6538)	5935-060A	Time Zero	1.18×10^4	1.2×10^5	N/A
		30 minutes	$< 1 \times 10^2$	8.1×10^4	>99.9%
		1 hour	$< 1 \times 10^2$	8.6×10^4	>99.9%
		2 hours	$< 1 \times 10^2$	1.25×10^5	>99.9%
		3hours	$< 1 \times 10^2$	1.32×10^5	>99.9%
		6 hours	$< 1 \times 10^2$	1.53×10^5	>99.9%
		24 hours	$< 1 \times 10^2$	$>3.00 \times 10^7$	>99.9%
	5935-060B	Time Zero	1.39×10^4	1.2×10^5	N/A
		30 minutes	1×10^2	8.1×10^4	99.9%
		1 hour	$< 1 \times 10^2$	8.6×10^4	>99.9%
		2 hours	$< 1 \times 10^2$	1.25×10^5	>99.9%
		3hours	$< 1 \times 10^2$	1.32×10^5	>99.9%
		6 hours	$< 1 \times 10^2$	1.53×10^5	>99.9%
		24 hours	$< 1 \times 10^2$	$>3.00 \times 10^7$	>99.9%
	5935-060C	Time Zero	2.36×10^5	1.2×10^5	N/A
		30 minutes	1×10^2	8.1×10^4	>99.9%
		1 hour	$< 1 \times 10^2$	8.6×10^4	>99.9%
		2 hours	$< 1 \times 10^2$	1.25×10^5	>99.9%
		3hours	$< 1 \times 10^2$	1.32×10^5	>99.9%
		6 hours	$< 1 \times 10^2$	1.53×10^5	>99.9%
		24 hours	$< 1 \times 10^2$	$>3.00 \times 10^7$	>99.9%

MRID #	Organism	Lot	Average Number of survivors	Carrier Population Count	Percent Reduction
492865-03	*Corynebacterium ammoniagenes*	5977-03F	$<1.00 \times 10^1$	9.77×10^6	>99.9%
		5977-03G	$<1.00 \times 10^1$		>99.9%
492865-04	*Escherichia coli O157:H7*	5977-03F	$<1.00 \times 10^1$	4.90×10^6	>99.9%
		5977-03G	$<1.00 \times 10^1$		>99.9%
492865-05	*Klebsiella pneumoniae*	5977-03F	$<1.00 \times 10^1$	3.47×10^7	>99.9%
		5977-03G	$<1.00 \times 10^1$		>99.9%
		5977-03H	$<1.00 \times 10^1$		>99.9%
	Staphylococcus aureus	5977-03F	$<1.00 \times 10^1$	4.07×10^6	>99.9%
		5977-03G	$<1.00 \times 10^1$		>99.9%
		5977-03H	$<1.00 \times 10^1$		>99.9%
	Pseudomonas aeruginosa	5977-03F	1.95×10^5	3.72×10^6	94.8%
		5977-03G	2.69×10^4		99.3%
		5977-03H	2.24×10^4		99.4%
492865-06	*Listeria monocytogenes*	5977-03F	$<3.55 \times 10^1$	5.62×10^7	>99.9%
		5977-03G	$<1.00 \times 10^1$		>99.9%
492865-07	*Salmonella enterica*	5977-03F	1.23×10^2	1.29×10^7	>99.9%
		5977-03G	1.41×10^2		>99.9%
492865-08	CA-MRSA Genotype USA 300	5977-03F	$<1.00 \times 10^1$	3.55×10^7	>99.9%
		5977-03G	$<1.00 \times 10^1$		>99.9%
492865-09	HA-MRSA	5977-03F	$<1.00 \times 10^1$	3.72×10^7	>99.9%
		5977-03G	$<1.00 \times 10^1$		>99.9%

MRID # 494455-01	Product Concentration	Observations (Number of Test Strips Showing Mold Growth/Number of Test Strips Exposed)			
		1 Week	2 Weeks	3 Weeks	4 Weeks
Bardac 20	440 ppm	0/10	0/10	2/10	6/10
	550 ppm	0/10	0/10	2/10	4/10
	660 ppm	0/10	0/10	0/10	2/10
	880 ppm	0/10	0/10	0/10	2/10
	1760 ppm	0/10	0/10	0/10	0/10
PMA (Control)	---	0/10	0/10	2/10	3/10
Untreated Control	---	0/10	3/10	8/10	10/10

MRID # 494455-01	Lot #	Exposure Time after 10 min Treatment	Percent Reduction
Escherichia coli (ATCC 4352)	906E	30 minutes	92.20%
		1 hour	96.88%
		6 hours	98.90%
		24 hours	>99.9%
	947E	30 minutes	94.44%
		1 hour	96.40%
		6 hours	99.14%
		24 hours	>99.9%
		30 minutes	92.84%

	7268	1 hour	95.92%
		6 hours	98.20%
		24 hours	>99.9%
Staphylococcus aureus (ATCC 6538)	906E	30 minutes	88.40%
		1 hour	95.90%
		6 hours	98.78%
		24 hours	>99.9%
	947E	30 minutes	86.82%
		1 hour	94.80%
		6 hours	99.02%
		24 hours	>99.9%
	7268	30 minutes	88.84%
		1 hour	94.88%
		6 hours	99.12%
		24 hours	>99.9%
Brevibacterium ammoniagenes (ATCC 6371)	906E	24 hours	99.99%
	947E	24 hours	99.99%
	7268	24 hours	99.99%

VI. CONCLUSION

1. The submitted efficacy data (MRID 492865-01 and MRID 492865-05) support the use of the products, Lonza Laundry Bacteriostat-Sanitizer when diluted 1:640 with 200 ppm hard water, and Bardac 20-10 (EPA Reg. No. 6836-65) when diluted 1:128 with 200 ppm hard water, as an effective laundry pre-soak sanitizer against *Enterobacter aerogenes* (ATCC 13048) and *Staphylococcus aureus* (ATCC 6538) at water to fabric ratio of 4:1 for a contact time of 5 minutes.

2. The submitted efficacy data (MRID 492865-02) support the use of the products, Lonza Laundry Bacteriostat-Sanitizer when diluted 1:2560 with 200 ppm hard water, and Bardac 20-10 (EPA Reg. No. 6836-65) when diluted 1:512 with 200 ppm hard water; for residual self-sanitizing in the laundry rinse cycle against *Klebsiella pneumoniae* (ATCC 4352) and *Staphylococcus aureus* (ATCC 6538), for commercial or industrial laundering operations (water to fabric ratio of 5:1) at 35°C and a contact time of 8 minutes.

3. The submitted efficacy data support the use of the products, Lonza Laundry Bacteriostat-Sanitizer when diluted 1:640 with 200 ppm hard water, and Bardac 20-10 (EPA Reg. No. 6836-65) when diluted 1:128 with 200 ppm hard water; as laundry sanitizer against the following microorganisms, in high efficiency application (water to fabric ratio of 4:1) at 35°C for a contact time of 5 minutes.

Corynebacterium ammoniagenes (ATCC 6872)	MRID 492865-03
Escherichia coli O157:H7 (ATCC 35150)	MRID 492865-04
Klebsiella pneumoniae (ATCC 4352)	MRID 492865-05
Staphylococcus aureus (ATCC 6538)	MRID 492865-05
Listeria monocytogenes (ATCC 19117)	MRID 492865-06
Salmonella enterica (ATCC 10708)	MRID 492865-07

Community Acquired Methicillin Resistant *Staphylococcus aureus* – CA-MRSA Genotype USA 300 (NARSA NRS 384) MRID 492865-08
Hospital Acquired Methicillin Resistant *Staphylococcus aureus* – HA-MRSA (NARSA NRS 382) MRID 492865-09

4. The submitted efficacy data (MRID 494455-01) support the use of the products, Lonza Laundry Bacteriostat-Sanitizer and Bardac 20-10 (EPA Reg. No. 6836-65) when diluted to 1760 ppm, as a 4 weeks Fabric Mildew Fungistatic in the laundry rinse cycle against *Aspergillus niger* (ATCC 6275) and *Penicillium glaucum*, in high efficiency application (water to fabric ratio of 4:1) at 35°C and a contact time of 10 minutes.

5. The submitted efficacy data (MRID 494455-01) support the use of the products, Lonza Laundry Bacteriostat-Sanitizer and Bardac 20-10 (EPA Reg. No. 6836-65) when diluted to 175 ppm, as a 24 hours minimum residual self-sanitizing in the laundry rinse cycle against *Escherichia coli* (ATCC 4352), *Staphylococcus aureus* (ATCC 6538), and *Brevibacterium ammoniagenes* (ATCC 6371), for commercial or industrial laundering operations (water to fabric ratio of 5:1) at 35°C and a contact time of 10 minutes

VII. LABEL

1. The proposed label claims **are acceptable** regarding the use of the products, Lonza Laundry Bacteriostat-Sanitizer when diluted 1:640 with 200 ppm hard water and Bardac 20-10 (EPA Reg. No. 6836-65) when diluted 1:128 with 200 ppm hard water, as effective laundry pre-soak sanitizer against *Enterobacter aerogenes* and *Staphylococcus aureus* at water to fabric ratio of 4:1 for a contact time of 5 minutes. These claims **are supported** by the applicant's data. **Registrant must add "High Efficiency Laundry Applications" to Laundry Pre-soak Sanitization" claims on the proposed label.**

2. The proposed label claims **are acceptable** regarding the use of the products, Lonza Laundry Bacteriostat-Sanitizer when diluted 1:640 with 200 ppm hard water and Bardac 20-10 (EPA Reg. No. 6836-65) when diluted 1:128 with 200 ppm hard water, as laundry sanitizer against the following microorganisms, in high efficiency application (water to fabric ratio of 4:1) at 35°C for a contact time of 5 minutes.

Corynebacterium ammoniagenes
Escherichia coli O157:H7 *[E. coli O157:H7]*
Klebsiella pneumoniae
Listeria monocytogenes
Salmonella enterica [Salmonella]
Staphylococcus aureus [Staph]
Staphylococcus aureus – Community Associated Methicillin-Resistant [CA-MRSA]
Staphylococcus aureus – Hospital Associated Methicillin resistant [HA-MRSA]

These claims **are supported** by the applicant's data. **Registrant must add "High Efficiency Laundry Applications" to Laundry Sanitization" claims on the proposed label**

3. The proposed label claims **are acceptable** regarding the use of the products, Lonza Laundry Bacteriostat-Sanitizer when diluted 1:2560 with 200 ppm hard water and Bardac 20-10 (EPA

Reg. No. 6836-65) when diluted 1:512 with 200 ppm hard water, as effective residual self-sanitizing laundry additive for final rinse cycle, in industrial or commercial application (water to fabric ratio of 5:1) against *Klebsiella pneumoniae* and *Staphylococcus aureus*, for a minimum rinse cycle time of 8 minutes. These claims **are supported** by the applicant's data.
Registrant must add "Commercial or Industrial Laundry Applications" to "Laundry Residual Self-Sanitization" claims on the proposed label.

4. The following proposed claims **are acceptable when revised** to reflect the following as supported by the applicant's data:

a. **PRESERVATION AGAINST MILDEW BY SOAKING, LAUNDRY MILDEW PRESERVATIVE SOAK or FABRIC MILDEW FUNGISTATIC** of Bardac 20-10 and Lonza Laundry Bacteriostat-Sanitizer, for no more than 4 weeks when diluted to **1760 ppm** in high efficiency application (water to fabric ratio of 4:1) and applied for 10 minutes. [*Aspergillus niger* (ATCC 6275) and *Penicillium glaucum*]

b. **RESIDUAL BACTERIOSTATIC** of Bardac 20-10 and Lonza Laundry Bacteriostat-Sanitizer, for a minimum 24 hours holding when used in industrial or commercial application (water to fabric ratio of 5:1) as laundry final rinse cycle at **175 ppm dilution** for 10 minutes contact.

Note: Previous Residual Bacteriostat and Laundry Mildew claims at **39 ppm** dilution of products, Bardac 20-10 and Lonza Laundry Bacteriostat-Sanitizer, must be removed from labels as those dilutions are not supported by the applicant's data.